AJTCAM AfroEthnoMedNet **Research Paper**

Afr. J. Traditional, **Complementary and Alternative Medicines** www.africanethnomedicines.net

ISSN 0189-6016©2008

PHYTOCHEMICAL, ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF THE ETHYLACETATE EXTRACT OF THE LEAVES OF *PSEUDOCEDRELLA KOTSCHYII*

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Abstract

Phytochemical screening was carried out on the ethylacetate portion of the ethanolic extract of the leaves of *Pseudocedrella kotschyii* and then evaluated for its analgesic (acetic acid-induced writhing) and anti-inflammatory (raw egg albumin-induced oedema) activities in mice and rats respectively. Phytochemical screening of the ethylacetate partition portion of ethanolic extract revealed the presence of flavonoids, glycosides and tannins as major chemical constituents. Alkaloids saponins, cardiac glycosides, steroids were not dictated in the extract. The ethylacetate extract (50 and 100 mg/kg i.p) exhibited significant activity ($p<0.05$) against acetic acid-induced writhing in a dose dependent manner. In the anti-inflammatory activity the ethylacetate extract (50 and 100 mg/kg i.p.) caused a slight effect against the raw egg albumin-induced oedema. The effect was however observed not to be dose dependent. All these effects were compared with standard drug piroxicam (20 mg/kg *i.p.*).

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Keywords: Acetic acid-induced writhing; Analgesic; Anti-inflammatory; Meliaceae; Oedema; Phytochemistry; *Pseudocedrella kotschyii*.

Introduction

Despite the progress that has occurred in recent years in the development of pain therapy, there is still need for effective and potent analgesic, especially for the treatment of chronic pain (Calixto *et al.,* 2000). Medicinal plants are believed to be important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). The research into plants with alleged folkloric use as pain-relievers should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic drugs (Elisabetsky *et al.,* 1995). One of the most important analgesic extract employed in clinical practice today continues to be the alkaloid morphine. Thousands of patients who suffer from intense and unrelenting pain, such as that resulting from cancer or injury, have to depend on morphine, despite its well-known side effects. This has renewed interest of the major pharmaceutical companies in medicinal plants as part of the search for new clinically useful extracts. Recently discovered analgesic substances include, alkaloids, flavonoids and terpenoids. Previous studies have demonstrated that systematically administered various flavonoids, including rutin, quercetin, pectolinarin and gossypin, all produce significant anti-nociception in different pain test (Calixto *et al.,* 2000).

Pseudocedrella kotschyii Harms, is a single species genus belonging to the family Meliaceae widely distributed in sub-Saharan zone of Central Africa; westerly limited in Senegal and easterly limited in Sudan and Uganda. It extends southwards to the savanna zone of southern Nigeria and also Congo-Kinshasa The plant is known locally as 'tona' or 'tonas' in Hausa, 'bodel' in Fulfulde, and 'emigbebi' or 'emigbegbe' in Yoruba languages (Dalziel, 1955). The roots and the leaves are used medicinally in Nigeria for rheumatism and other

diseases. The bitter bark is used in Togo in infusions for gastrointestinal, febrile and rheumatic conditions (Dalziel, 1955). A decoction of root-bark and leaves is used as a sitz bath for piles. The pulped leafy twigs are used as a medicine for stomach pains and headache (Kerharo and Bouquet, 1950). There is no scientific report or verification of the use of this plant in the treatment of pain and rheumatic conditions. Previous phytochemical investigation of stem bark of *Pseudocedrella kotschyii* results in the isolation of saponins, sterols and/or triterpenes and tannins. The structures of the compounds isolated were determined chemically and spectroscopically to be 24-mthylenecycloartanol, cyloeucalenol and pseudrelone A, B, C β-sitosterol (Ekong and Olagbemi, 1967; Ekong *et al.,* 1968; Delaveau *et al.,* 1979). This study was therefore aimed at investigating possible antinociceptive and anti-inflammatory effects of the ethylacetate portion of the ethanolic extract of the leaves of *Pseudocedrella kotschyii*.

Experimental

Plant Material

The leaves of *Pseudocedrella kotschyii* were collected in March 2004 in Samaru village, Zaria, Kaduna State, Nigeria. The plant material was identified by Muhammad Musa and U. A. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria. A voucher specimen (No. 900243) was deposited in the Herbarium for further reference.

Extraction procedure

The air-dried powdered leaves of *Pseudocedrella kotschyii* (750 g) was defatted with petroleum ether (60-80 ºC) and then extracted with ethanol (95 %) using a soxhlet device. Solvent was removed under reduced pressure to afford 13.66 % w/w of residue that was coded EE. 30 g of the ethanolic extract were shaken in water (400 ml) and residue insoluble matter removed by filtration. The aqueous filtrate was partition successively with ethyl acetate and n-butanol to afford an ethyl acetate soluble portion (12.98 % w/w) and n-butanol soluble portion (21.90 % w/w) which was coded as ET and BT respectively. The ethylacetate soluble portion (ET) was screened for analgesic and anti-inflammatory activities using the methods described under the sections analgesic and anti-inflammatory activities respectively.

Phytochemical screening

The ethylacetate soluble portion (ET) used for the pharmacological screening was tested for the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids/ or terpenoids according to the standard procedure (Sofowora, 1993; Trease and Evans, 1997).

Animals

Swiss albino mice (20-25 g) and adult Wister rats (180-250 g) of either sex were used through out this study. The animals were kept under standard laboratory conditions in the presence of light and at room temperature, fed on standard feeds (Excel Feeds Plc, Zaria) and provided with drinking water *ad libitum.*

Drugs

Piroxicam (Pfizer) was purchased from a Pharmacy retail store in Samaru-Zaria, Nigeria and formaldehyde from BDH reagent (Poole, UK). All drugs were freshly prepared to the desire concentration with distilled water just before use. The extract was also freshly prepared using distilled water.

Acute Toxicity Studies

The crude ethanolic extract (EE) of the leaves of *Pseudocedrella kotschyii* was subjected to acute toxicity tests (Lorke, 1983) to determine the LD_{50}.

Analgesic activity
Acetic acid-induced writhing test

The response to *i.p*. injection of an acetic acid solution is a contraction of the abdominal muscle and stretching of the hind limbs; induced according to the method described by (Winter *et al.,* 1963). Mice were randomly divided into four groups (n=6) and were administered 0.06% acetic acid (10 ml/kg body weight *i.p.*). The number of abdominal constrictions was registered over 15 minutes, starting 5 minutes after acetic acid injection. Mice were treated intraperitoneally as follows: group 1: distilled water (10 ml/kg as negative control); groups 2 and 3: ET (50 and 100 mg/kg body weight) and group 4: piroxicam (20 mg/kg body weight), 30 minutes before acetic acid administration respectively. The results were expressed as percentages in relation to the distilled water pre-treated control group. A significant reduction in the number of writhing in the group treated with ET compared with the control was considered to be a positive analgesic response.

Anti-inflammatory Activity
Raw egg albumin-induced oedema test

The Ugo Basile model LE: 7150 was used to measure the anti-inflammatory effect. Acute inflammation was induced by the sub-planter administration of 0.1 ml of raw egg albumin in the left hind paw of the rats. Rats were randomly divided into four groups (n = 6) and were treated intraperitoneally as follows: group 1: distilled water (10 ml/kg as negative control); groups 2 and 3: ET (50 and 100 mg/kg body weight) and group 4: piroxicam (20 mg/kg body weight), 30 minutes before administration of the raw egg albumin. The paw volume was measured for 2 hours at 20 minutes intervals after administration of the raw egg albumin using plethysmometre (Genè 1998).

Statistical Analysis

The results were expressed as mean ± SEM. Statistical significant differences between means were evaluated using Student's t-test and results were regarded as significant at $P<0.05$.

Results and Discussions

The phytochemical screening of ET of *Pseudocedrella kotschyii* used in the pharmacological tests has revealed the presence of tannins, glycosides and flavonoids major chemical constituents. Alkaloids, saponins, cardiac glycosides, steroids were not detected in the extract. The LD_{50} of the crude ethanolic extract (EE) in mice was determined to be 1131 mg/kg body weight (i.p). Figure 1 shows the antinociceptive effects of ET of *P. kotschyii* on acetic acid-induced abdominal constriction in mice. The extract at doses 50 and 100 mg/kg body weight *i.p*. reduced the number of abdominal constrictions by 51.57 % and 64.04 % respectively. Piroxicam, as a reference drug, produced 76.62 % (Figure 1). All the values were significant ($P< 0.05$) compared with the negative control.

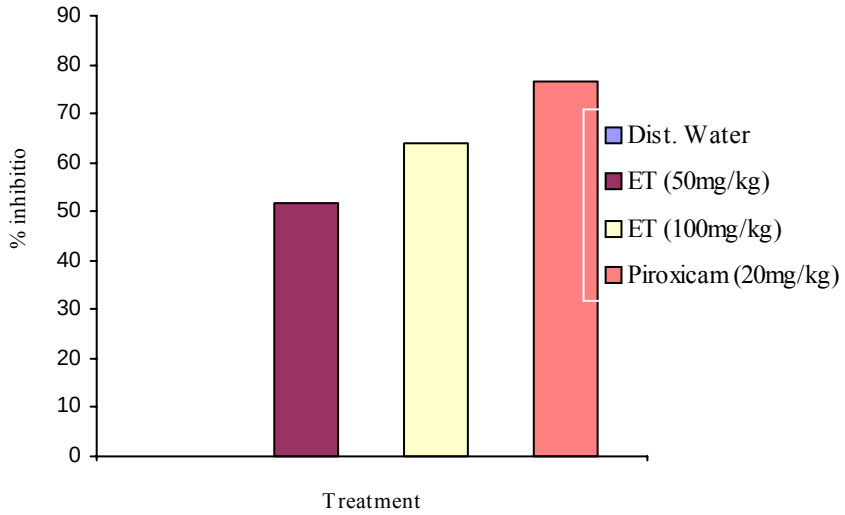

Fig. 1: Percentage Inhibition of ET of *P. kotschyii* and Piroxicam on Acetic acid induced writhing in Mice

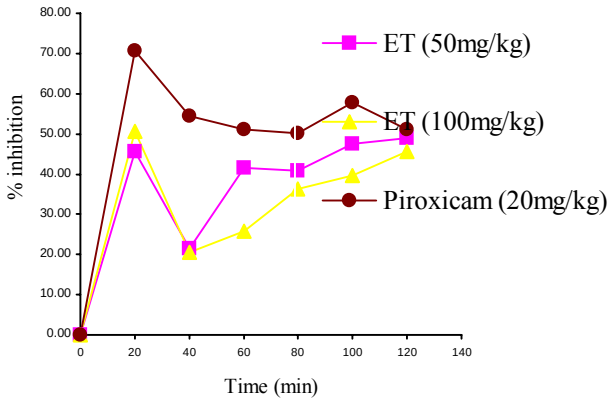

Fig. 2: Percentage inhibition of ET of *P. kotschyii* and Piroxicam on raw egg albumin-induced oedema in Rat

Antinociceptive effects of the extract at the doses tested revealed that it significantly reduced the number of writhes in mice signifying that it possesses peripherally mediated activities. These effects were observed to be dose dependent but with no significant ($P>0.05$) difference between the two test doses (Figure 1). Although this test is a non-specific model it is widely used for the evaluation of peripheral anti-nociceptive activity (Collier *et al.*, 1968). Also called the abdominal constriction response, it is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in the other method like the tail-flick test (Bentley *et al.*, 1981; Bentley *et al.*, 1983). Local peritoneal receptors are postulated to be partly involved in abdominal constriction response (Derardt *et al.*, 1980). The method has been associated with prostanoids in general, e.g. increase level of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids (Levini *et al.*, 1984) as well as lipoxygenase products by some researchers (Dhara *et al.*, 2000; Famacy, 1983). Therefore, the results of the acetic acid-induced writhing strongly suggest that the mechanism of action of this extract may be linked to lipoxygenase and/or cyclo-oxygenases.

The anti-inflammatory activity of leaves extract of *P. kotschyii* against acute paw oedema (induced by raw egg albumin) is presented in Figure 2 and the results show that ET cause a slight inhibition of albumin-induced oedema over a periods of 120 minutes. This effect however, did not appear to be dose-dependent. Maximum inhibitory effect (48.94 %) was observed at a dose of 50mg/kg, 120 minutes after drug treatment although the value was statistically significant ($p<0.05$) compared with the negative control.

The method used in determining the anti-inflammatory activity is useful in detecting activity in acute inflammation. The co-existence of both anti-nociceptive and anti-inflammatory effects seen with this extract is well defined for various non-steroidal anti-inflammatory drugs (NSAIDS) particularly Salicylates and their derivatives. It is therefore interesting that the extract behave like NSAIDS in this study which correlates well with the traditional application of the plant. Analgesic and anti-inflammatory effects have already been observed in flavonoids as well as tannins (Ahmadiani *et al.*, 1998; Ahmadiani *et al.*, 2000). It is therefore, possible that both the anti-nociceptive and anti-inflammatory effects observed with this extract may be attributed to its flavonoids and/or tannins contents shown to be present during phytochemical studies. Indeed, published biological effects of polyphenols such as flavonoids and tannins appear to justify some of the traditional, folkloric uses of the leaves of the plant in the control and/or management of painful and inflammatory conditions. Although the exact chemical constituents of the plant that are responsible for the analgesic and anti-inflammatory effects still remain speculative.

Conclusions

In conclusion, the ethylacetate soluble portion of ethanolic extract of the leaves of *Pseudocedrella kotschyii* possesses significant antinociceptive and anti-inflammatory effects in laboratory animals at the doses investigated. The results support the traditional claim for the use of this plant in the treatment of rheumatic conditions. More detailed phytochemical studies are necessary to identify the active principles and exact mechanism of action. Further studies are in fact currently under way to isolate and characterize the active principle(s) of the ethylacetate soluble portion of the ethanolic extract.

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